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DISCLAIMER

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ABSTRACT

Since radiation fields of space contain many-fold more protons than high atomic number, high energy (HZE) particles, cells in astronaut crews will experience on average several proton hits before an HZE hit. Thus radiation regimes of proton exposure before HZE particle exposure simulate space radiation exposure, and measurement of the frequency of neoplastic transformation of human primary cells to anchorage-independent growth simulates in initial step in cancer induction. Previously our group found that exposure to 20 cGy 1 GeV/n protons followed within about 1 hr by a HZE ion (20 cGy 1 GeV/n Fe or Ti ions) hit gave about a 3-fold increase in transformation frequency ([1]). To provide insight into the H-HZE induced increased transformation frequencies, we asked if split doses of the same ion gave similar increased transformation frequencies. However, the data show that the split dose of 20 cGy plus 20 cGy of either H or HZE ions gave about the same effect as the 40 cGy uninterrupted dose, quite different from the effect of the mixed ion H + HZE irradiation. We also asked if lower proton doses than 20 cGy followed 15 minutes later by 20 cGy of HZE ions gave greater than additive transformation frequencies. Substantial increases in transformation levels were observed for all proton doses tested, including 1 cGy. These results point to the signal importance of protons in affecting the effect of space radiation on human cells.

INTRODUCTION

Radiation fields in space contain protons and high atomic number, high energy (HZE) particles. Calculations indicate that during a three-year mission to Mars, the nucleus of a cell in a space traveler who is shielded by 0.4 g/cm^2 of aluminum will be hit by about 400 protons and about 0.3 HZE particles [2, 3]. In such a radiation field, the probability is high that any cells hit by an HZE particle had experienced several previous proton hits within recent cellular memory. A major uncertainty in projecting risks of cancer for space travelers is the lack of biological data [4], especially for the combined effects of protons and HZE particles [1].

Zhou et al. recently investigated the effects of sequential dual beam irradiations on transformation to soft-agar growth of primary human cells [1]. They found that irradiation of cells with 20 cGy of 1 GeV/n H ions followed within about one hour by 20 cGy of 1 GeV/n Fe ions increased the transformation frequency by about three-fold. Strikingly, the same ions in the opposite sequence gave transformation levels in about the same range as that expected for the additive effect of those doses of the two ions administered separately. Further, longer interbeam intervals also gave only additive transformation levels [1, 5].

However, their report did not address two significant factors: First, the total dose of 40 cGy was delivered as one 20 cGy dose of protons followed later by 20 cGy of HZE particles, in essence a split dose exposure. Thus it was not clear whether the increased transformation observed in the dual beam experiments was unique to protons and HZE particles, or if split doses of a single ion could also affect transformation frequencies. Further, Zhou et al. used one dose of each ion in their dual beam experiments. Although

20 cGy of a HZE particle correspond to approximately 1 hit/nucleus, 20 cGy of protons represents many fold higher fluxes of protons than cells would encounter within a short time. They did not determine whether lower doses of protons (as the first beam in a sequential, dual beam experiment) could also affect transformation frequencies.

We have investigated these aspects of the dual beam irradiations. We first found that split doses of the same ion (administered at the same interval as that yielding maximum transformation increase in the dual ion beam experiments) did not increase transformation frequencies. Instead, the transformation levels were similar to those obtained at the same total dose of each ion. We also irradiated primary cells with lower doses of 1 GeV/n protons (0-20 cGy), followed 15 minutes later by 20 cGy of 1 GeV/n Fe ions, and determined the transformation frequencies. Even at the lowest proton dose used (1 cGy), the transformation levels were higher than that expected for the additive effect of those doses of protons used plus 20 cGy Fe ions.

MATERIALS AND METHODS

Human cells

Primary human fibroblast cultures initiated in this laboratory were grown in a 1:1 mixture of MCDB-153 medium and Dulbecco's Modified Eagle Medium (DMEM, Gibco, Carlsbad, CA) containing per l, 4.9 g sodium bicarbonate plus 15% fetal bovine serum (FBS, Hyclone, Logan, UT) as previously described [1]. Cells were grown without antibiotics, were periodically tested (Bionique, Saranac Lake, NY) and certified free of mycoplasma. Cells and media were handled in yellow-illuminated rooms [6].

Irradiations

BNL's NASA Space Radiation Laboratory (NSRL) provided heavy charged particles [Fe (1.005 GeV/n); Ti (1.007 GeV/n)] and protons (H, 1 GeV/n). The linear energy transfer values (LETs) for these ions are: for 1.005 GeV/n Fe, 151.3 keV/μm (μm, micrometer); for 1.007 GeV/n Ti, 108.1 keV/μm; for 1 GeV/n H, 0.22 keV/μm. Dose rates were < 1 Gy/min. The NSRL Physics Dosimetry Group provided dosimetry and developed beam switching procedures. Cells in medium were irradiated at room temperature; controls were sham-treated using the same procedure except for irradiation. The medium in each flask was replaced by fresh medium, and the flasks were incubated at 37^{o}C.

Anchorage-independence and survival determinations

Anchorage-independent growth was assessed as previously described [7], with modification of media and agar layers as described [1]. In brief, 10^5 cells were plated in a 3 ml soft agar layer over a 9 ml agar base in 60 mm dishes, and each dish was then

scored immediately for cell clumps that could grow to be mistaken for colonies. After 3 weeks growth, colonies of >~50 cells were counted. Clonogenic survival was determined by plating cells at low densities on solid surfaces or in permissive agarose medium, then incubating and counting colonies as above.

RESULTS

We first asked if dual exposures of human cells to two beams of the same ion species also affected the transformation frequency. In this split-dose experiment, we used the ions, energies, and optimum inter-exposure interval that gave the maximal increase in transformation seen by Zhou et al. [1]. The doses and energies were 20 cGy of 1 GeV/n H ions plus 20 cGy of the same ion, or 20 cGy of 1.007 GeV/n Ti ions plus 20 cGy of the same ion.

Figure 1 shows the results of such experiments. The filled circles show the yields of soft-agar transformants per survivor as a function of dose for single exposures of H ions alone (▲) or a single exposure of Ti ions alone (●). In addition, we exposed cells to 20 cGy H ions followed 15 min later by an additional exposure of 20 cGy H ions, corresponding to a total of 40 cGy. The open triangles show that the experimental results for the yield of soft agar colonies/survivor are in the range as the experimental data for irradiation with one continuous exposure of 40 cGy of these ions. In parallel samples, we exposed cells to 20 cGy Ti ions followed 15 min later by an additional exposure of 20 cGy Ti ions, again corresponding to a total dose of 40 cGy. The open circles show that the experimental results for the yield of soft agar colonies/survivor are in the range as the experimental data for irradiation with one continuous exposure of 40 cGy of Ti ions. These results are strikingly different from the results of sequential dual beam exposures (20 cGy H ions followed 15 min later by 20 cGy Ti ions), shown as the open square in Figure 1. These data thus indicate that the source of the increase in transformants in the sequential H + HZE ion dual beam experiments is not simply the splitting of the total

radiation dose into two fractions separated by a short interval (15 min for the data shown).

We also studied the generality of the ion doses for increased transformation by the H + HZE sequential dual beams. Zhou et al. used 20 cGy of HZE ions, corresponding to about 1 hit per nucleus of these cells, on the basis of Poisson statistics. Since most space radiation exposures will produce ~ 1 hit/nucleus within cellular memory, this dose is a reasonable approximation of space radiation conditions. They also used the same dose of H ions, 20 cGy, but they did not report whether lower proton doses also increased the transformation levels. Since most space radiation exposures of protons will be many-fold lower than that dose, the question of the effect of lower H ion exposures on transformation frequencies is significant.

To test the effect of lower doses of protons on transformation yields in dually-irradiated cells, we irradiated cells with increasing doses of 1 GeV/n protons (in the 0-20 cGy range), then 15 min later, with a single dose of 20 cGy of 1.005 GeV/n Fe ions. Companion samples were exposed to the same doses of H ions (but no subsequent Fe ions). Figure 2 shows the results of such experiments. The closed circles show the transformation frequencies measured in cells exposed to a single dose of H ions, in the range of 0-20 cGy. The closed triangles show the frequencies obtained for cells exposed to the dose as plotted (0-20 cGy) of H ions, and then 15 min later to 20 cGy of Fe ions. (The level of transformants/survivor induced by 20 cGy of Fe ions alone (▲) is plotted on the vertical axis at the position corresponding to 0 dose of H ions (0 on the x- axis), and the background transformation level for cells receiving no radiation exposure is plotted (●) at the 0 dose position as well.) The results clearly show that even at the

smallest H dose tested (1 cGy), increased yields of transformants/survivor were produced in the H + Fe dually-irradiated cells.

DISCUSSION

The data in Figure 1 clearly show that split doses of the same ion beam –whether H ion or HZE particle—have substantially different effects on human cell neoplastic transformation levels than sequential irradiations of H ions followed shortly by HZE ions. This difference holds even for the same doses of these ions at the same time intervals between exposures. Zhou et al. showed that the inter-beam timing is important: the window of increased transformation was about 1 hr for both the H-Fe and H-Ti pairs [1]. Further, they found that the order of the beams is critical: H ion exposure followed shortly by HZE exposure yielded about a three-fold increase in transformants/survivor, whereas the reverse order of beams resulted in only an additive effect on the transformation levels. No significant protection or decrease in transformation was observed in either sequence of irradiation. These results clearly point out the interesting and significant role of proton exposure in altering cellular responses to subsequent HZE ion exposure.

Since protons apparently play a major role in radiation damage to cells induced by dual beam irradiations, it is important to know whether H ion doses lower than the 20 cGy used in these experiments also increase transformation frequencies by subsequent HZE exposures. Figure 2 shows that this is indeed the case. Simple addition of the experimental transformation levels produced by 20 cGy H ions plus that of 20 cGy Fe ions gives an expected total of ~ 38 expected transformants/10^5 survivors, compared with the actual experimental value of 105 transformants/10^5 survivors for the sequential dual beam irradiations of 20 cGy of H ions and Fe ions each and a 15 minute inter-beam

interval. Similar calculations can be made at other H ion doses in the 0-20 cGy range. Clearly the experimental transformation frequencies in the dually-irradiated cells are much higher than that expected from simple additive values of the H ion dose chosen plus 20 cGy Fe ions (approximately 1 hit/cell nucleus) for these cells.

Space travelers on a 3-year mission to Mars will experience approximately 1000:1 proton:HZE hits. Thus the probability is high that a cell that is hit by a HZE particle will have previously been hit by several protons within cellular memory. Further, during solar proton events, the potential is substantial for unshielded (or incompletely shielded) cells to be exposed to even higher proton fluxes. Thus determination of the effects of dual beam irradiations is important to provide insight into space radiation risks for astronaut crews. The current studies provide additional insight into the origin of the increased human cell transformation by combined exposures to protons and HZE particles.

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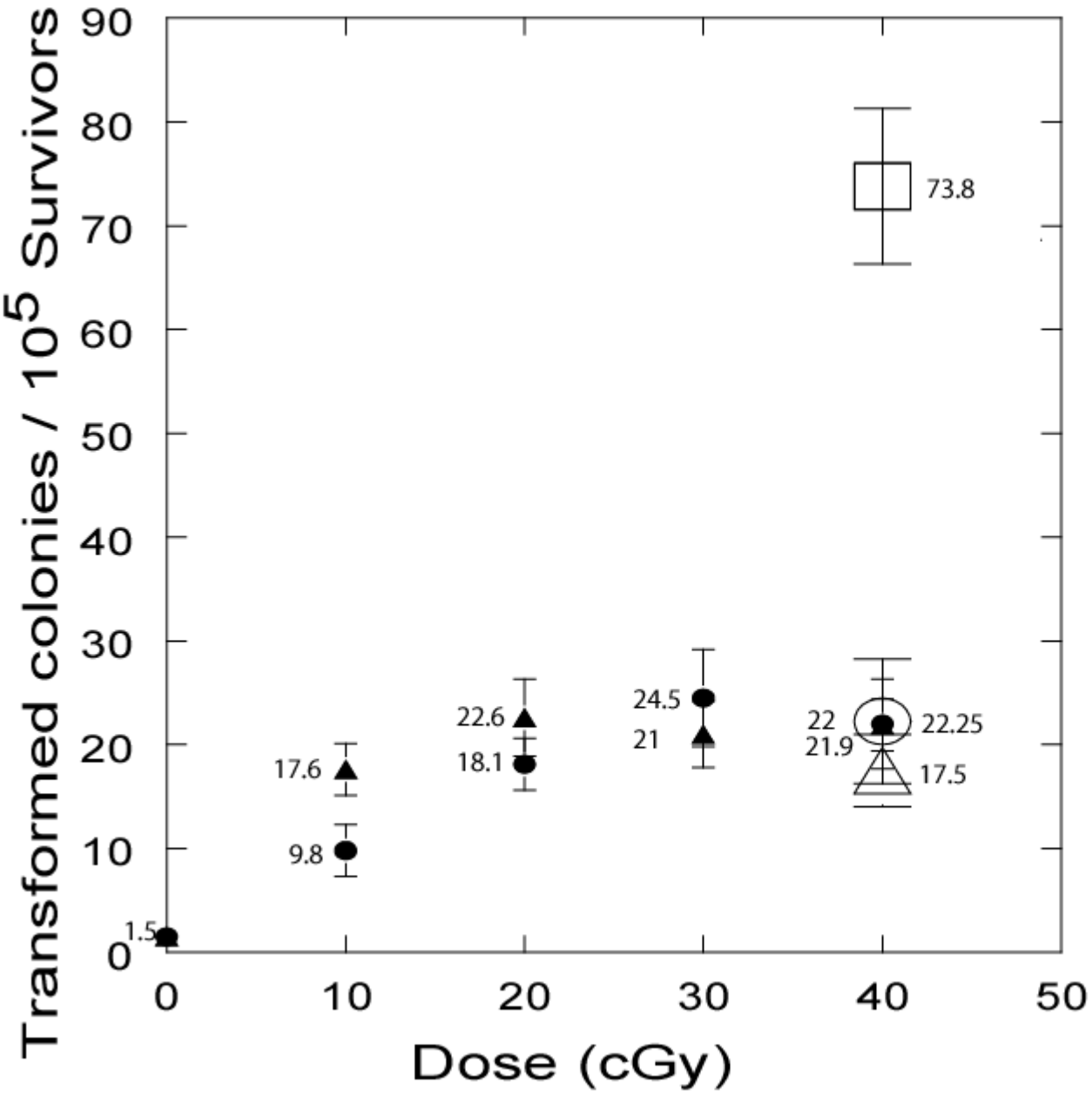
FIGURE LEGENDS

Figure 1.

Soft agar colonies per 10^5 survivors in cells irradiated with a single dose of 1 GeV/n H ions (▲), a single dose of 1.007 GeV/n Ti ions alone (●), or a total dose of 40 cGy of H ions split into two equal 20 cGy exposures spaced by 15 min (△), or a total dose of 40 cGy of Ti ions administered in two equal 20 cGy exposures spaced by 15 min (○). The transformation level for cells exposed to 20 cGy H ions and 15 min later to 20 cGy Fe ions is also shown (□). Data points are the averages of at least 6 replicate measurements; error bars, SD.

Figure 2

Soft agar colonies per 10^5 surviving colonies in cells irradiated with increasing doses of 1 GeV/n H ions alone (●), or with the same doses of H ions followed 15 min later by 20 cGy Fe ions (▲). The ▲ point plotted at 0 H dose corresponds to the result of cell exposure to 20 cGy Fe ions alone. The ● point plotted at the 0 H dose shows the background level of transformants/10^5 survivors for unirradiated (sham control) cells. Data points, the averages of at least 6 replicate determinations.


Transformed colonies / 10^5 Survivors
90
80
70
60
50
40
30
20
10
0
0
10
20
30
40
50
Dose (cGy)
1.5
9.8
17.6
18.1
22.6
21
24.5
21.9
22
22.25
17.5
73.8

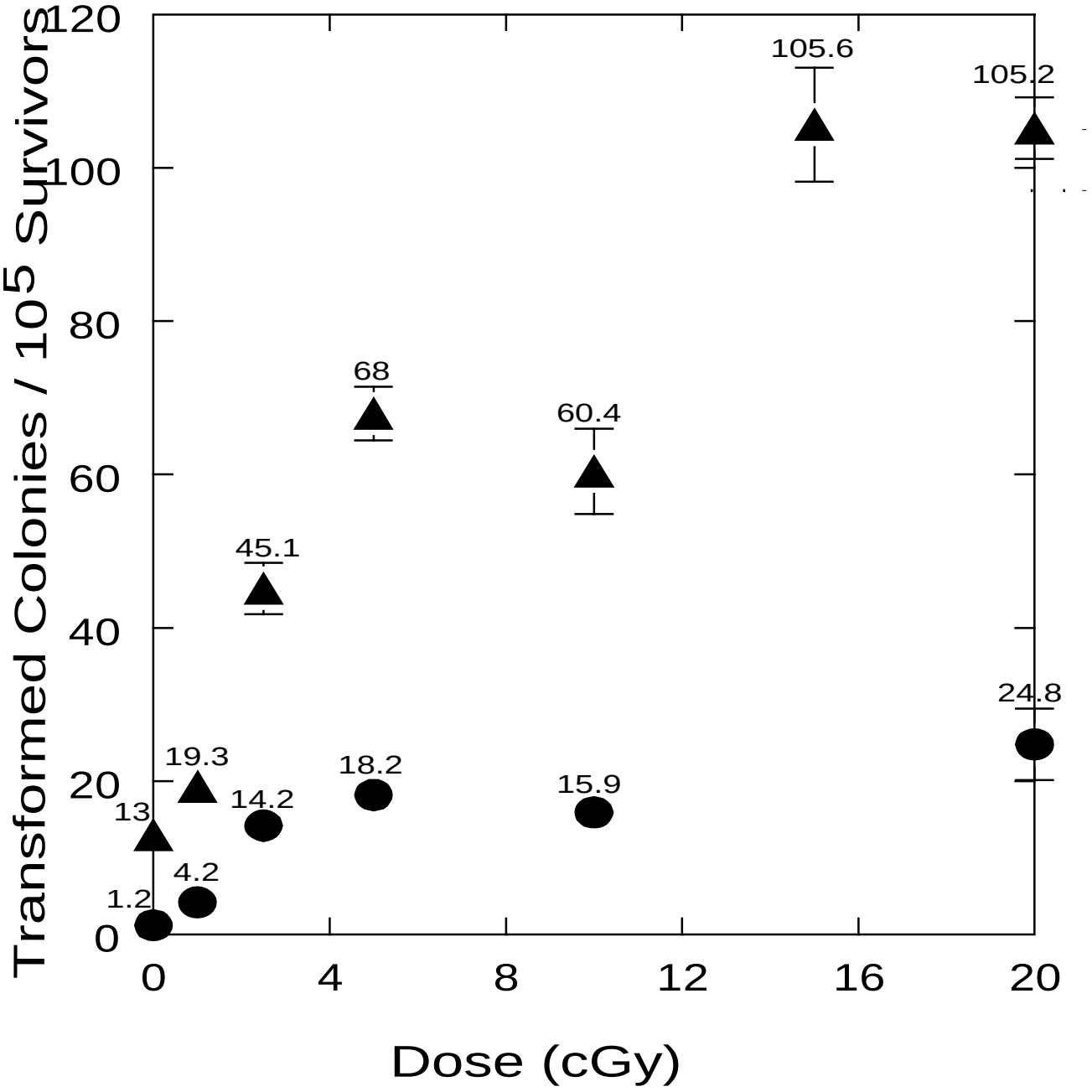

Transformed Colonies / 10^5 Survivors
Dose (cGy)
0
20
40
60
80
100
120
0
4
8
12
16
20
105.6
105.2
68
60.4
45.1
24.8
19.3
18.2
15.9
14.2
13
4.2
1.2

Table 1. Irradiation procedures.

Irradiation Procedure		Result
A. Single ion beam irradiation		
Cells —H or HZE→	Soft Agar Assay	Dose response (Transformants/survivor)
B. Sequential ion beam irradiation		
Cells —H→ 15 ' Interval —HZE→	Soft Agar Assay	Super-additive Level of Transformants/survivor
C. Split dose irradiation (single ion beam)		
Cells —H→ 15 ' Interval —H→	Soft Agar Assay	Additive Level of Transformants/survivor